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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Roy Curtiss, III and Steve A. Tinge

#24 DGJ 9/1/98

Serial No.: 08/473,~~780~~ 789-PCO Group Art Unit: 1641

Filed: June 7, 1995 Examiner: V. Ryan

For: *RECOMBINANT BACTERIAL VACCINE SYSTEM WITH ENVIRONMENTALLY LIMITED VIABILITY*

Assistant Commissioner for Patents
Washington, D.C. 20231

TRANSMITTAL OF APPEAL BRIEF

Sir:

In response to the Office Action finally rejecting claims 1-4, 8-14, 16, 20, 23, 24,27-29, and 37, mailed December 23, 1997, enclosed is an Appeal Brief, in triplicate, along with a check in the amount of $155.00 which is the fee for filing of an Appeal Brief by a small entity. It is believed that no additional fee is required with this submission. However, should a fee be required, the Commissioner is hereby authorized to charge any additional

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fees to Deposit Account No. 01-2507. To facilitate this process, a duplicate of this Transmittal of Appeal Brief is enclosed.

Respectfully submitted,

Robert A. Hodges
Reg. No. 41,074

Date: July 22, 1998

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I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Teresa R. Spratt

Date: July 22, 1998

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants: Roy Curtiss III and Steven A. Tinge

Serial No.: 08/473,789 Group Art Unit: 1641

Filed: June 7, 1995 Examiner: V. Ryan

For: *RECOMBINANT BACTERIAL VACCINE SYSTEM WITH ENVIRONMENTALLY LIMITED VIABILITY*

Assistant Commissioner for Patents
Washington, D.C. 20231

APPEAL BRIEF

Sir:

This is an appeal from the final rejection of claims 1-4, 8-14, 16, 20, 23, 24, 27-29, and 37 in the Office Action mailed December 23, 1997, in the above-identified patent application. A Notice of Appeal was mailed on May 22, 1998. A check in the amount of $155.00 for the filing of this Appellants' Brief for a small entity is also enclosed.

(1) REAL PARTY IN INTEREST

The real party in interest of this application is Megan Health, Inc.

(2) RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to Appellants, the undersigned, or Appellants' assignee which directly affects, which would be directly affected by, or which would have a bearing on the Board's decision in this appeal.

(3) STATUS OF CLAIMS ON APPEAL

Claims 1-38 are pending. Claims 5-7, 15, 17-19, 21-22, 25, 26, 30-36, and 38 have been withdrawn from consideration as being drawn to a non-elected invention. Claims 1-4, 8-14, 16, 20, 23, 24, 27-29, and 37 are on appeal. The text of each claim on appeal, as amended, is set forth in the Appendix to this Appeal Brief.

(4) STATUS OF AMENDMENTS

An amendment after final rejection was mailed on April 22, 1998. The Advisory Action mailed May 8, 1998, indicated that this amendment was not entered.

(5) SUMMARY OF THE INVENTION

The present invention is a microbial cell having an Environmentally Limited Viability System (ELVS) such that the cell is viable in a permissive environment and non-viable in a non-permissive environment (see page 5, lines 14-19). The ELVS achieves this environmentally specified viability using two components, an essential gene and a lethal gene

(see page 5, lines 16-19; claims 1 and 27). Essential genes and lethal genes are specifically limited in the claims, and defined in the specification (see pages 11-17, especially pages 11 and 16), to refer to mutually exclusive genes having mutually exclusive effects. As recited in the claims, an essential gene is a gene whose expression is essential to the viability of the cell (see page 11, lines 15-16). A lethal gene is a gene whose expression is lethal to the cell (see page 16, lines 7-8). Thus, a lethal gene according to the claims cannot be an essential gene, and an essential gene cannot be a lethal gene. The expression of the lethal gene and the expression of the essential gene, as recited in the claims, are also mutually exclusive. The essential gene is regulated such that the essential gene is expressed in the permissive environment but is not expressed in the non-permissive environment (see page 11, lines 16-19). The lethal gene is regulated such that the lethal gene is expressed in the non-permissive environment but is not expressed in the permissive environment (see page 16, lines 8-10). This distinction between essential genes and lethal genes is important for assessing the relevance of the publications cited in the rejections.

Permissive environments at a temperature of about 37°C and non-permissive environments at a temperature of less than about 30°C, as recited in claims 2 and 28, are described on page 20, lines 8-21. Permissive environments inside an animal and non-permissive environments outside of an animal, as recited in claims 3, 23, and 29, are described on page 20, lines 8-21. Localization of essential genes and lethal genes on a vector, as recited in claim 4, is described on page 12, lines 7-8, and page 17, lines 11-12,

respectively. An ELVS vector having two lethal genes and vector pMEG-104, as recited in claims 8 and 9, is described on page 24. Use of a host cell that is a gram-negative enteric bacterium of the genus *Escherichia* or *Salmonella*, as recited in claims 10-12, is described on pages 30-31. Regulation of expression of the essential gene by the expression product of a regulatory gene, as recited in claims 13, 14, and 37, is described on page 19, lines 3-10. An environmentally regulated replication gene required for replication of the ELVS vector, as recited in claim 16, is described from page 17, line 24, to page 18, line 8. Inclusion of an expression gene encoding a desired expression product, as recited in claims 20 and 24, is described on page 35, lines 13-18.

(6) ISSUES ON APPEAL

The issues presented on appeal are (1) whether claims 1-4, 8-14, 16, 20, 23, 24, 27-29, and 37 should be rejected under 35 U.S.C. § 112, first paragraph, (2) whether claims 1-3, 10-13, 16, 20, 23, 24, 27, 28, and 37 should be rejected under 35 U.S.C. § 102(b) as anticipated by Gerdes *et al.*, *Proc. Natl. Acad. Sci. USA* 83:3116-3120 (1986), and (3) whether claims 1-3, 8, 10-14, 16, 20, 27, 28, and 37 should be rejected under 35 U.S.C. § 102(b) as anticipated by Gerdes *et al.*, *EMBO Journal* 5(8):2023-2029 (1986).

(7) GROUPING OF CLAIMS

The claims on appeal do not stand or fall together. The claims on appeal should be grouped as follows. In regard to the rejection under 35 U.S.C. § 102(b) over Gerdes *et al.*, *Proc. Natl. Acad. Sci. USA* 83:3116-3120 (1986), the claims fall into three groups of separately patentable claims: (1) claims 1-3, 10-13, 23, 27, 28, and 37; (2) claim 16; and (3) claims 20 and 24. In regard to the rejection under 35 U.S.C. § 102(b) over Gerdes *et al.*, *EMBO Journal* 5(8):2023-2029 (1986), the claims fall into five groups of separately patentable claims: (1) claims 1-3, 10-12, 27, 28, and 37; (2) claim 8; (3) claims 13 and 14; (4) claim 16; and (5) claim 20. Separate bases for patentability of each of these groups are argued below.

(8) ARGUMENTS

(i) Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-4, 8-14, 16, 20, 23, 24, 27-29, and 37 stand rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification is enabling only for the use of some *Salmonella* strains as a vaccine. Appellants respectfully traverse this rejection.

Appellants initially note that the rejection is based on a misinterpretation of the claimed cells. The claimed Environmentally Limited Viability System (i.e. the regulated essential and lethal genes recited in the claims) is **not** a bacterial attenuation system. Rather, the environmentally regulated expression of the lethal and essential genes results in cell

viability in the permissive environment and cell **non-viability** in the non-permissive environment. Attenuation of virulent bacteria is completely different and represents a feature that can be **combined** with the claimed Environmentally Limited Viability System. Where appropriate, the claimed Environmentally Limited Viability System can be embodied in a host cell that has been attenuated. Such attenuation is well known and is thoroughly described in the specification.

It is not clear what is the basis for the present rejection. For example, the passage on page 4, lines 7-10, of the Office Action mailed December 23, 1997, appears to object to the administration of virulent bacteria (such as *Salmonella*) as a vaccine composition. First, the claims do not require administration of a virulent strain of bacteria as a vaccine composition. Second, it is not clear how such administration relates in any way to enablement of the present claims. In this regard, Appellants note that even if attenuation were required in some applications, the state of the art and the extensive guidance in the specification for producing attenuated bacteria provide the requisite enablement for such attenuation. Appellants submit that the reasoning of the present rejection is unfounded, and that, as a consequence, no *prima facie* case of lack of enablement has been established.

Appellants also note that the claimed cells need not produce a robust immune response (although many of the claimed cells will have this ability). Only claims 23-26 and 30-35 recite the use of the cells as a vaccine or a method of inducing immunoprotection. The specification (page 39) defines vaccine as an agent used to stimulate the immune system

of a living organism so that an immune response occurs. Significantly, the specification states that *preferred* vaccines are sufficient to stimulate the immune system of a living organism so that protection against future harm is provided, indicating that the vaccine need not induce immunoprotection. Only claims 30-35 require this level of immune response. While the rejection implies that some mutations used in the Environmentally Limited Viability System (that is, *asd* and *pur* mutations) will render the claimed cells incapable of producing immunoprotection. However, as described in the specification, mutations in essential genes such as *asd* and *pur* are *complemented* by an environmentally regulated form of the essential gene. Thus, the claimed cells are not, in fact, Asd- or Pur- (when *asd* or *pur* is used as the essential gene) since the environmentally regulated versions of the genes provide the necessary function. Thus, even if an *asd* or *pur* mutant *Salmonella* strain exhibited reduced immunogenicity (as asserted in the rejection), such a result is not relevant to the claimed cells since the claimed cells remain Asd+ or Pur+ (in the permissive environment).

(ii) Rejections Under 35 U.S.C. § 102

1. Claims 1-3, 10-13, 16, 20, 23, 24, 27, 28, and 37 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Gerdes *et al.*, *Proc. Natl. Acad. Sci. USA* 83:3116-3120 (1986). Appellants respectfully traverse this rejections.

Gerdes *et al.* (PNAS) disclose (page 3119, second column, and Fig. 3) *E. coli* containing a *hok* gene linked to λP_R which is regulated by the temperature-sensitive λCI_{857}

repressor. The *hok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *hok* gene produces a highly toxic gene product which causes rapid cell death. Thus, *hok* can be considered a lethal gene as defined in the specification. Gerdes *et al.* (PNAS) also separately discloses a *sok* gene regulated by the temperature-sensitive λCI_{857} repressor. The *sok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *sok* gene regulates expression of the *hok* gene. Thus, the *sok* gene can be considered a regulatory gene as defined in the specification. Gerdes *et al.* (PNAS) fail to disclose any environmentally regulated *essential* gene.

The claimed cells require both an environmentally regulated lethal gene **and** an environmentally regulated essential gene. Since Gerdes *et al.* (PNAS) fails to disclose or suggest such a regulated essential gene, Gerdes *et al.* (PNAS) fails to disclose each and every feature of the claimed cells. Accordingly, Gerdes *et al.* (PNAS) fails to anticipate the claimed cells and method.

To the extent that the present rejection is based on the assertion that the *sok* gene is an essential gene, Appellants note the following. As disclosed by Gerdes *et al.* (PNAS), the *sok* gene is a regulatory gene of *hok* gene expression. Attached as Exhibit 1 is a copy of Franch and Gerdes, *Molecular Microbiology* 21:1049-1060 (1996), which describes the mechanism of this regulation. The *sok* gene produces an RNA that is an anti-sense RNA to RNA transcribed from the *hok* gene. When both the *sok* and *hok* genes are expressed, *sok*

RNA prevents translation of *hok* RNA (and thus prevents production of the lethal *hok* protein). The *hok* RNA is much more stable that the *sok* RNA. Thus, when production of both *sok* and *hok* RNA ceases (such as when the *hok*-bearing plasmid is lost), the *sok* RNA is degraded first allowing translation of *hok* RNA to produce *hok* protein and cell death. This relationship between *sok* RNA and *hok* gene expression makes the *sok* gene a **regulatory** gene of the *hok* gene.

Appellants submit that a regulatory gene regulating expression of a lethal gene is clearly not intended to be an essential gene within the claimed Environmentally Limited Viability System. The claimed ELVS is intended to operate with separate essential genes and lethal genes, each with a role to play. Regulatory genes are separately contemplated as a separate component (see page 19, lines 3-10, in the specification). Although any regulatory gene (as defined in the specification) of a lethal gene might technically fit the definition of an essential gene (since the cell may die when the regulatory gene fails to repress expression of the lethal gene), such regulatory genes are clearly not intended to be considered essential genes within the context of the claimed cells. That is, when read in light of the specification, the claimed essential gene does not encompass regulatory genes of the claimed lethal gene. Appellants assert that the *sok* gene as disclosed by Gerdes *et al.* (PNAS) is a regulatory gene, not an essential gene, since it regulates expression of the *hok* gene and is not otherwise separately "essential" to the viability of the cell. Thus, Gerdes *et al.* (PNAS)

fails to disclose cells as presently claimed. Accordingly, Gerdes *et al.* (PNAS) fails to anticipate claims 1-3, 10-13, 16, 20, 23, 24, 27, 28, and 37.

Gerdes *et al.* (PNAS) also fails to disclose an environmentally regulated replication gene. Claim 16 requires an environmentally regulated replication gene the expression of which is required for replication of the vector. Thus, for this additional reason, Gerdes *et al.* (PNAS) fails to disclose all of the limitations of claim 16. Accordingly, for this additional reason, Gerdes *et al.* (PNAS) fails to anticipate claim 16.

Gerdes *et al.* (PNAS) also fails to disclose any expression gene or the expression of any desired expression product. Claim 20 requires that the cell include an expression gene encoding a desired expression product. Thus, for this additional reason, Gerdes *et al.* (PNAS) fails to disclose all of the limitations of claim 20. Accordingly, for this additional reason, Gerdes *et al.* (PNAS) fails to anticipate claim 20.

2. Claims 1-3, 8, 10-14, 16, 20, 27, 28, and 37 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Gerdes *et al.*, *EMBO Journal* 5(8):2023-2029 (1986). Appellants respectfully traverse this rejection.

Gerdes *et al.* (EMBO) disclose (page 2024, second column, and Fig. 1) *E. coli* containing a *hok* gene linked to λP_R which is regulated by the temperature-sensitive λCI_{857} repressor. The *hok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *hok* gene produces a highly toxic gene product which causes rapid cell death. Thus, *hok* can be considered a lethal gene. Contrary

to the implication of the present rejection, Gerdes *et al.* (EMBO) fails to disclose a temperature regulated *sok* gene. Accordingly, Gerdes *et al.* (EMBO) fails to disclose any environmentally regulated essential gene.

The claimed cells require both an environmentally regulated lethal gene **and** an environmentally regulated essential gene. Since Gerdes *et al.* (EMBO) fails to disclose or suggest such a regulated essential gene, Gerdes *et al.* (EMBO) fails to disclose each and every feature of the claimed cells. Accordingly, Gerdes *et al.* (EMBO) fails to anticipate the claims 1-3, 8, 10-14, 16, 20, 27, 28, and 37.

Gerdes *et al.* (EMBO) also fails to disclose a vector having two lethal genes. Claim 8 requires that the vector in the cell have two lethal genes. Thus, for this additional reason, Gerdes *et al.* (EMBO) fails to disclose all of the limitations of claim 8. Accordingly, for this additional reason, Gerdes *et al.* (EMBO) fails to anticipate claim 8.

Gerdes *et al.* (EMBO) also fails to disclose any regulatory gene the product of which regulates expression of an essential gene. Claims 13 and 14 require a regulatory gene the product of which regulates expression of an essential gene. Thus, for this additional reason, Gerdes *et al.* (EMBO) fails to disclose all of the limitations of claims 13 and 14. Accordingly, for this additional reason, Gerdes *et al.* (EMBO) fails to anticipate claims 13 and 14.

Gerdes *et al.* (EMBO) also fails to disclose an environmentally regulated replication gene. Claim 16 requires an environmentally regulated replication gene the expression of

which is required for replication of the vector. Thus, for this additional reason, Gerdes *et al.* (EMBO) fails to disclose all of the limitations of claim 16. Accordingly, for this additional reason, Gerdes *et al.* (EMBO) fails to anticipate claim 16.

Gerdes *et al.* (EMBO) also fails to disclose any expression gene or the expression of any desired expression product. Claims 20 and 24 require that the cell include an expression gene encoding a desired expression product. Thus, for this additional reason, Gerdes *et al.* (EMBO) fails to disclose all of the limitations of claims 20 and 24. Accordingly, for this additional reason, Gerdes *et al.* (EMBO) fails to anticipate claims 20 and 24.

(9) SUMMARY AND CONCLUSION

The claims on appeal are fully enabled at least because attenuation of host bacteria is not required for the claimed cells and because attenuation of virulent bacteria, even if it were required for the claimed cells, is a well established technology, thoroughly described in the specification, which can be practiced by those of skill in the art without the need for undue experimentation. The claims on appeal are not anticipated by either Gerdes *et al.* (PNAS) or Gerdes *et al.* (EMBO) since neither publication discloses each of the limitations of the claims. In particular, neither Gerdes *et al.* (PNAS) nor Gerdes *et al.* (EMBO) disclose an environmentally regulated essential gene.

For the foregoing reasons, Appellants submit that the claims 1-4, 8-14, 16, 20, 23, 24, 27-29, and 37 are patentable.

Respectfully submitted,

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Date: July 22, 1998

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Teresa R. Spratt

Date: July 22, 1998

APPENDIX

Claims on appeal:

1. An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment.

2. The cell of claim 1 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

3. The cell of claim 1 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

4. The cell of claim 1 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector.

8. The cell of claim 4 wherein the vector has two lethal genes.

9. The cell of claim 8 wherein the vector comprises pMEG-104.

10. The cell of claim 1 wherein the cell is a gram-negative bacterium.

11. The cell of claim 10 wherein the gram-negative bacterium is an enteric bacterium.

12. The cell of claim 11 wherein the genus of the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.

13. The cell of claim 1 wherein expression of the essential gene is regulated by an expression product of a regulatory gene.

14. The cell of claim 13 wherein the expression product of the regulatory gene inhibits expression of the essential gene and is expressed or active only in the non-permissive environment.

16. The cell of claim 4 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

20. The cell of claim 1 further comprising an expression gene wherein the expression gene encodes a desired expression product.

23. The cell of claim 1 for use as a vaccine, wherein the cell is viable when in the an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the

cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

24. The cell of claim 23 further comprising an expression gene wherein the expression gene encodes a desired expression product.

27. A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment;

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment.

28. The method of claim 27 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

29. The method of claim 27 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

37. The cell of claim 13 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

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